# Effects of growth implants and energy substrate infusion on enzymes responsible for marbling and subcutaneous fat deposition in beef cattle

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## Introduction

USDA Quality Grade is used as a predictor of eating quality of beef products. The primary factors used to determine USDA Quality Grade are maturity (age) and the amount of intramuscular fat (marbling) distributed in beef muscles. Beef cuts with higher levels of marbling have a greater probability of being more tender, juicy and flavorful than cuts with low levels of marbling. The National Beef Quality Audit conducted in 1995 revealed that only 12% of the U.S. beef supply had enough marbling to grade USDA Average Choice (Modest degree of marbling) or higher, while the present and future demand for higher quality beef is actually 28% of supply. Since the beef industry is currently unable to meet the demand for this higher quality product, beef producers, processors and retailers are not able to obtain their full economic potential. Using expected progeny differences (EPDs), it is possible to select bulls that will sire offspring with the desirable levels of IM fat (marbling), without excessive subQ fat. It is because of this issue that animal scientists have initiated research to characterize the mechanisms involved in marbling development in beef cattle. This could eventually lead to nutritional or management strategies that will allow cattle to obtain their genetic potential for intramuscular fat and increase the value of the carcass.

### Background: Growth enhancing implants

Growth enhancing implants are used in the beef industry to increase the rate and efficiency of gain of feedlot cattle, which considerably reduces the cost of production for feedlot operators. Research, however, has revealed that certain growth implant strategies reduce the amount of marbling deposited by cattle, and also decrease the tenderness of beef products. A recent review by Oklahoma State University of 37 implant studies showed that when implanted cattle are sold on a carcass grade and yield basis, average value per head is reduced by $11.00 due to the reduction in quality grade as a result of implanting. For Indiana feedlot producers, this translates into an estimated loss of potential agricultural dollars of $1.8 million (165,000 head of cattle on feed on January 1, 1999 x $11.00/head reduced value). Until recent years, there has been virtually no research by scientists attempting to elucidate the mechanism by which implants are reducing carcass quality in beef cattle. Burch et al. (1982) analyzed enzyme activities in liver, subcutaneous fat and muscle (intramuscular fat) of sheep receiving either zero or one implant during the finishing period. They reported

decreased activity of fatty acid synthetase in liver and subcutaneous fat in implanted lambs compared to non-implanted lambs. They did not detect any significant differences in enzyme activity in intramuscular fat samples between treatments. These researchers concluded that implants influence adipose tissue enzymes. However, their study only included 3 animals per treatment, which may affect the ability to detect significant differences.

### Background: Energy substrates for fat development

Beef cattle use energy substrates produced from the digestion process as precursors (building blocks) for fat production. These energy substrates include propionate, acetate, lactate, and glucose. Recent research using in-vitro techniques (in a test tube) has found that marbling and subcutaneous fat are influenced differently by the different energy substrates. The ability to determine which energy substrates increase marbling, without increasing subcutaneous fat would allow for development of nutritional or technological strategies to increase the production of the particular substrate in the animal. Management practices to either increase the production of specific substrates, or the uptake of substrates by marbling fat cells could then be developed so that Indiana beef producers could produce a higher quality, more consistent beef product.

## Project Objectives

The objectives of the two research projects were to determine how common management practices in the beef industry influence marbling and subcutaneous fat accretion in cattle so that strategies could be developed to improve the profitability and sustainability of Indiana's cattle industry. The specific objective of the growth implant project was to establish the mechanism by which certain implant strategies negatively affect marbling, and lower the perceived value and palatability of beef products. The primary objective of the substrate infusion project is to determine which of the energy substrates resulting from the ruminant digestion process are the primary precursors for fat deposition in the subcutaneous or intramuscular fat sites. Subsequently, new feeding strategies could be developed to enhance the production and uptake of the substrate(s) by the intramuscular fat cell so that more marbling would be deposited.

## Materials and Methods

### Growth Implant Study

To determine the effects of growth enhancing implants and implant strategies on marbling deposition in beef cattle, 21 Angus or Angus x Simmental steers were randomly assigned to one of 3 implant treatments. Treatments included; 1) non-implanted controls (Control), 2) Component E-S day 1 of the feeding period followed by Revalor-S on day 63 (Comp/Rev), and 3) Revalor-S day 1, followed by a second Revalor-S 63 days later (Rev/Rev). Treatment two was considered a moderately aggressive implant strategy (i.e. increase daily gain and feed

efficiency moderately compared to no implant), while treatment three would be an aggressive implant program (i.e. dramatically increase daily gain and feed efficiency compared to no implant). Steers were fed a high concentrate (corn) diet similar to diets fed in typical Indiana feedlots. All steers were harvested at the Purdue University Meats Laboratory. Immediately after the animals were harvested, a section of subcutaneous fat and the underlying muscle was removed from the loin region. Using aseptic techniques, the subcutaneous fat was separated from the muscle tissue, sliced, flash-frozen in liquid nitrogen, and stored in a –80° C freezer for further analysis. The same procedure was followed for the muscle tissue, which contained the intramuscular fat (marbling). From the subcutaneous and intramuscular fat samples collected after harvest, activities of key enzymes responsible for fat deposition in the two fat depots were analyzed to determine which enzymes may be negatively or positively affected by implant treatments. The enzymes analyzed included NADP malate dehydrogenase (malic enzyme), glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. The metabolic reactions catalyzed by these three enzymes all produce nicotinamide adenine dinucleotide phosphate (NADPH). Therefore, the concept of the assays to measure activity of these three enzymes is that higher activity will result in the production of higher levels of NADPH. NADPH is required for fatty acid synthesis in fat tissue as it contributes hydrogen to the metabolic reactions. Protein concentration of each sample was analyzed so that enzyme activity results could be standardized.

### Substrate Infusion Study

In order to determine which of the major energy substrates resulting from the ruminant digestive process are involved in subcutaneous and intramuscular fat development, 30 jugularly cannulated market ready heifers were infused with one of five substrates (saline control, acetate, propionate, glucose, or lactate). Rates of infusion of the energy substrates were determined individually for each heifer based on their ad libitum intake during the four days preceding infusion. During infusion, cattle were fed 90% of their previous ad libitum intake, while the remaining 10% of energy intake was supplied by the appropriate infused energy substrate. Control heifers were fed ad libitum during the infusion period so that their supply of energy for fat synthesis was the normal combination of energy substrates derived from the digestive process. Heifers were infused for 48 hours and then harvested at the Purdue University Meats Laboratory. Immediately after animals were harvested, a section of subcutaneous fat and the underlying muscle was removed from the $5^{th}$ through $8^{th}$ rib region. Using aseptic techniques, subcutaneous fat and intramuscular fat were dissected, sliced, flash-frozen in liquid nitrogen, and stored in at –80° C for further analysis. Activities of NADP malate dehydrogenase (malic enzyme), glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were measured in the fat samples. Protein concentration of each sample was analyzed so that enzyme activity results could be standardized.

## Results and Discussion

### Substrate Infusion Study

Activities of each enzyme are reported in two ways; 1) nmoles of NADPH produced/minute/gram (g) of wet tissue, and 2) nmoles of NADPH produced/minute/milligram (mg) protein. Average activity of malic enzyme, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase, in intramuscular adipose tissue following substrate infusion is shown in Table 1. When expressed on a per gram of wet tissue basis, infusion with glucose upregulated the activity of malic enzyme compared to infusion with lactic acid (98.48 vs. 47.27). However, there was not a statistical difference between treatments for malic enzyme activity when expressed as activity/minute/mg protein. Activities of 6PGDH and G6PDH were not affected in intramuscular adipose tissue following infusion with any of the energy substrates used in this experiment (Table 1). Similarly, energy substrate infusion had no effect on activities of the three enzymes in subcutaneous adipose tissue (Table 2).

Previous research reported that glucose infusion in cattle increased activity of malic enzyme in subcutaneous adipose tissue compared to saline infusion (Prior and Scott, 1980). However, cattle in that study weighed approximately 800 lbs. and were fed pelleted alfalfa hay diets, whereas in our study, cattle were approximately 1150 lbs. and were fed a high concentrate finishing diet. Therefore, differences in both physiological stage of maturity and diet energy density between the two studies may account for the conflict of results obtained for malic enzyme in subcutaneous fat. Additionally, the study of Prior and Scott, 1980 did not analyze enzyme activity in intramuscular adipose tissue. Results from our study indicate that when compared to ad libitum fed control cattle, activities of three enzymes that supply NADPH for fat synthesis are not influenced by infusion of acetate, propionate, glucose or lactate. Previous in vitro research revealed that acetate is the primary contributor of carbon atoms for fatty acid synthesis in subcutaneous fat, while glucose is the primary precursor for marbling (Smith and Crouse, 1984). Our results revealed that infusion of cattle with these two substrates did not up-regulate activity of the NADPH producing enzymes in either fat depot. Because of various metabolic interactions that occur within the body, our study would seem to better represent actual phenomena taking place in cattle compared to an in-vitro (test tube) study. It is conceivable that other enzymes involved in fat synthesis that were not measured in this study may be influenced by one of the infused substrates. However, our results show that supplying more of a specific energy substrate during the late finishing phase will not differentially increase malic enzyme, 6-phosphogluconate dehydrogenase, or glucose-6-phosphate dehydrogenase in marbling or subcutaneous fat.

## Growth Implant Study

Effects of different implant strategies on activities of malic enzyme, 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase in intramuscular and subcutaneous fat are shown in Tables 3 and 4, respectively. No statistical differences in enzyme activities were found in either fat source between implanted and non-implanted steers. A review of 37 implant studies detected reductions of 24% in marbling and 14.5% in the number of carcasses grading choice with implants (Duckett et al., 1996). Therefore, in our study, we expected to see a reduction in enzyme activity for enzymes responsible for marbling deposition because reductions in activities of lipogenic enzymes are highly correlated with reductions in fat accretion. However, finding no differences in lipogenic enzyme activity in our study supports the recent results of Duckett et al. (1999). Those researchers reported that implants reduce the total fat percentage in ribeye samples and increase ribeye muscle area. When calculated on a per-steak weight basis, which would account for the increased ribeye area, fat percentages did not differ between implanted and non-implanted cattle. In summary, when our results are combined with those of Duckett et al. (1999), we conclude that implants decrease marbling scores and percentage of carcasses grading USDA Choice by a dilution effect with the increase in ribeye size rather than down-regulating enzymes responsible for fat deposition.

## Impact

Determining which energy substrate(s) increase the activity of enzymes involved in marbling deposition could lead to development of nutritional strategies. Identification of specific feed ingredients or additives that increase the production, or cellular uptake of certain precursors, is the next step to enhancing the value of Indiana cattle. Development of successful nutritional strategies to increase the amount of marbling deposited in feedlot cattle would allow Indiana's beef producers, processors, and retailers to receive higher economic returns by enabling them to sell a higher quality, more palatable beef product to consumers. Additionally, because we have provided further evidence of the mechanism by which growth implants influence carcass quality grades, we can now develop implant strategies for producers to minimize this detrimental affect.

## Literature Cited

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## Table 1. Effect of energy substrate infusion on intramuscular adipose en

| Item | Saline | Propionate | Acetate | DL-Lactate | Glucose |
|---|---|---|---|---|---|
| n | 5 | 5 | 6 | 5 | 6 |
| Malic enzyme | | | | | |
| U[1]/g tissue | 85.45[ab] | 87.58[ab] | 66.67[ab] | 47.27[b] | 98.48[a] |
| U/mg protein | 4.17 | 4.23 | 3.14 | 3.03 | 4.37 |
| 6PGDH[2] | | | | | |
| U/g tissue | 242.73 | 233.33 | 220.71 | 223.64 | 265.91 |
| U[2]/mg protein | 4.17 | 3.39 | 3.27 | 4.37 | 3.54 |
| G6PDH[3] | | | | | |
| U/g tissue | 640.61 | 476.06 | 521.46 | 497.27 | 612.63 |
| U[2]/mg protein | 10.57 | 6.84 | 7.87 | 8.83 | 8.48 |

[1]Units = nmoles NADPH produced per minute.
[2]6-phosphogluconate dehydrogenase.
[3]Glucose-6-phosphate dehydrogenase.
[ab]Means in a row without a common superscript differ (P<0.05).

## Table 2. Effect of energy substrate infusion on subcutaneous adipose en

| Item | Saline | Propionate | Acetate | DL-Lactate | Glucose |
|---|---|---|---|---|---|
| n | 5 | 5 | 6 | 5 | 6 |
| Malic enzyme | | | | | |
| U[1]/g tissue | 29.70 | 39.09 | 38.13 | 27.88 | 41.16 |
| U/mg protein | 4.87 | 6.13 | 6.06 | 4.77 | 5.74 |
| 6PGDH[2] | | | | | |
| U/g tissue | 423.64 | 347.58 | 359.34 | 410.30 | 415.91 |
| U/mg protein | 20.58 | 18.97 | 18.76 | 19.56 | 21.15 |
| G6PDH[3] | | | | | |
| U/g tissue | 1078.48 | 876.06 | 868.94 | 1291.21 | 988.13 |
| U/mg protein | 53.96 | 47.24 | 44.21 | 61.51 | 51.42 |

[1]Units = nmoles NADPH produced per minute.
[2]6-phosphogluconate dehydrogenase.
[3]glucose-6-phosphate dehydrogenase.

## Table 3. Effect of implant program on intramuscular adipose enzyme activity

| Item | Control | Comp/Rev[1] | Rev/Rev[2] | SEM | P-value |
|---|---|---|---|---|---|
| n | 7 | 7 | 7 | | |
| Malic enzyme | | | | | |
| U[3]/g tissue | 42.21 | 30.95 | 50.76 | 7.30 | 0.16 |
| U/mg protein | 0.132 | 0.096 | 0.154 | 0.023 | 0.19 |
| 6PGDH[4] | | | | | |
| U/g tissue | 130.74 | 137.01 | 134.42 | 18.51 | 0.97 |
| U/mg protein | 0.408 | 0.424 | 0.441 | 0.068 | 0.94 |
| G6PDH[5] | | | | | |
| U/g tissue | 282.68 | 329.87 | 335.71 | 79.45 | 0.88 |
| U/mg protein | 0.885 | 1.029 | 1.132 | 0.268 | 0.81 |

[1]Comp/Rev = Component-ES day 1/Revalor-S day 63.
[2]Rev/Rev = Revalor-S day 1/Revalor-S day 63.
[3]Units = nmoles NADPH produced per minute.
[4]6-phosphogluconate dehydrogenase.
[5]Glucose-6-phosphate dehydrogenase.

## Table 4. Effect of implant program on subcutaneous adipose enzyme activity

| Item | Control | Comp/Rev[1] | Rev/Rev[2] | SEM | P-value |
|---|---|---|---|---|---|
| n | 7 | 7 | 7 | | |
| Malic enzyme | | | | | |
| U[3]/g tissue | 74.03 | 74.46 | 93.51 | 12.37 | 0.46 |
| U/mg protein | 2.34 | 1.95 | 2.27 | 0.28 | 0.59 |
| 6PGDH[4] | | | | | |
| U/g tissue | 641.99 | 648.70 | 697.84 | 72.91 | 0.84 |
| U/mg protein | 20.52 | 16.97 | 17.36 | 1.73 | 0.31 |
| G6PDH[5] | | | | | |
| U/g tissue | 1306.71 | 1535.50 | 1725.54 | 151.56 | 0.18 |
| U/mg protein | 42.56 | 41.28 | 43.11 | 4.83 | 0.96 |

[1]Comp/Rev = Component-ES day 1/Revalor-S day 63.
[2]Rev/Rev = Revalor-S day 1/Revalor-S day 63.
[3]Units = nmoles NADPH produced per minute.
[4]6-phosphogluconate dehydrogenase.
[5]Glucose-6-phosphate dehydrogenase.